

## ORIGINAL ARTICLE/ORIJİNAL ÇALIŞMA

FULL PAPER

TAM MAKALE

## THE MATRIX EFFECT OF TUNA AND MAHI-MAHI ON BIOGENIC AMINE DETECTION

Taylor DOLE<sup>1</sup>, Stephen KOLTUN<sup>1</sup>, Shirley M. BAKER<sup>2</sup>,  
Renee M. GOODRICH-SCHNEIDER<sup>1</sup>, Maurice R. MARSHALL<sup>1</sup>, Paul J. SARNOSKI<sup>1</sup>

<sup>1</sup> University of Florida, Institute of Food and Agricultural Sciences, Department of Food Science and Human Nutrition, Gainesville, Florida, USA

<sup>2</sup> University of Florida, Institute of Food and Agricultural Sciences, School of Forest Resources and Conservation, Gainesville, Florida, USA

Received: 19.02.2016

Accepted: 10.03.2016

Published online: 11.03.2016

Corresponding author:

Paul J. SARNOSKI, University of Florida, Institute of Food and Agricultural Sciences, Department of Food Science and Human Nutrition, Gainesville, Florida, 32611, USA

E-mail: [psarnoski@ufl.edu](mailto:psarnoski@ufl.edu)

### Abstract:

Method of standard addition (MSA) is used to account for and quantify matrix effect. In this study, MSA was used to analyze the matrix effect of mahi-mahi and tuna in respect to biogenic amine detection. Two different detection methods were used in conjunction with the MSA procedure: a colorimetric strip that detects volatile biogenic amines and a histamine-specific ELISA (enzyme-linked immunosorbant assay), which detects histamine in the liquid phase. Trained sensory experts were utilized to grade mahi-mahi and tuna. The grading system acted as a measure of fish quality. A biogenic amine cocktail was created to act as the standard spike for the MSA procedure. It contained histamine and other biogenic amine compounds of varying volatility that are present in nature. The ELISA-MSA showed a clear correlation between increase in histamine and a lower quality sample (higher numerical grade). The colorimetric strips did not have a clear correlation between biogenic amine content and grade. Comparing the two methods demonstrates that the matrix effect of the tuna and mahi-mahi is likely more prominent with gas phase compounds than liquid phase compounds.

**Keywords:** Biogenic amines, ELISA, Histamine, Mahi-Mahi, Tuna

## Introduction

Histamine and other biogenic amines are found in food products and can have toxicological implications. These compounds are known to affect various biological systems including the respiratory, gastrointestinal, cardiovascular, hematological, and immunological systems (Prester, 2011). Other biogenic amines of toxicological interest include tyramine and cadaverine. These compounds compete with histamine for oxidation by histamine metabolizing enzymes, thus increasing the toxicity of histamine itself (Halász, 1994). Histamine is a major food safety concern as the compound is heat-stable and thus not affected by any cooking process (Ahmed, 1991). The primary illness associated with the presence of histamine in fish is scombroid poisoning.

Scombroid poisoning has a number of symptoms ranging from nausea, vomiting, GI distress, and many signs of an anaphylactic attack like swelling and itching. Although rare, these symptoms can be severe enough to result in death. The symptoms appear quickly, normally within an hour of consuming the spoiled fish (CDC, 2013). Scombroid poisoning was initially associated with *Scombroidea* fish (including tuna and mackerel) but other vectors have been identified outside of this classification including mahi-mahi. Tuna and mahi-mahi are the two most implicated causes of scombroid poisoning in the US (CDC, 2013).

The principle seafood product attributed to scombroid poisoning is spoiled fish, mainly tuna species that naturally contain high levels of histidine (a precursor of histamine). The conversion of this amino acid to the toxic biogenic amines is carried out by certain bacterial species including *Morganella morgani*, *Klebsiella pneumonia*, *Hafnia alvei*, *Pseudomonas putrefaciens*, and *Clostridium perfringens* that contain the histidine decarboxylase enzyme (Ashie, 1996; Hidalgo and others, 2013). Moderate temperature and low pH favor the formation of histamine (Hidalgo and others, 2013). Histamine does not exist in even distribution within the fish muscle. Instead, it is found in highest concentration in areas around the dorsal and interior muscles (Yamanaka, 1982).

Imported fish (particularly mahi-mahi) have been the source of many scombroid poisoning outbreaks in the United States. The higher ambient water temperature of the tropics and poor handling conditions likely contribute to the formation of

histamine (Ahmed, 1991). This helps to explain how non-scombroid species can be vectors for histamine poisoning.

A matrix effect occurs when the components of the sample other than the analyte interfere with the determination of the target analyte's concentration. To account for the matrix effect and produce an accurate quantification of the analyte, the method of standard additions (MSA) is often used. This method consists of measuring the analyte within the sample before and after the addition of known amounts of analyte standard (or "spikes") are added. The responses of the spiked and unspiked samples are plotted and regressions are used to determine the analyte concentration in the original sample (Saxberg, 1979). The FDA often recommends this method for measuring furan content in food samples (Altaki, 2007). However, MSA has mostly been used for liquid samples or liquid extracts. The MSA method should be able to be used for gas phase detection methods, given that gas phase quantitation has been extensively studied. Pinho and others (2002) have shown how volatile measurement of solid-liquid matrices can be done using solid-phase microextraction (SPME) to measure volatile free fatty acids in cheeses.

The aim of this study was to determine the concentration of biogenic amines in tuna and mahi-mahi using a MSA procedure via volatile measurement of biogenic amines and histamine. The hypothesis was that the MSA procedure would allow for the determination of matrix effect of the fish tissue along with the quantification of biogenic amines and histamine in both mahi-mahi and tuna samples.

## Materials and Methods

### Samples and Sensory Grading System

Frozen mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) filets were obtained from waters in the South Pacific near Indonesia, and were evaluated by up to five FDA and National Marine Fisheries Service (NMFS) sensory experts during a grading session according to the standard sensory grading system of the FDA ORA Laboratory Manual (FDA, 2013). The fish filets were received from the FDA/NMFS frozen, individually vacuum packed, and shipped overnight on dry ice. Each filet had been evaluated

by sensory experts and graded on a 1 through 7 scale, representing the quality of the fish. This grading system is based on olfaction. Each filet was rated on a 100-point scale and ranked into one of seven grades. The cutoff between a pass and fail sample is a score of 50, with all scores above 50 being a “pass” (grades 1-4) and all scores below 50 being a “fail” (grades 5-7). The same grading scale was used in the work of Pivarnik et al. (2001).

### Storage and Preparation of Fish Samples

Upon arrival, the fish filets were stored at  $-20^{\circ}\text{C}$ . For each grade of fish, seven samples were defrosted overnight at room temperature ( $\sim 20^{\circ}\text{C}$ ). The defrosted filets were cut into smaller pieces by hand, and then homogenized twice using a blender (Total Blend Classic WildSide, Blendtec, Orem, UT). In order to assure uniformity of sample, food grade dye was added to the sample to confirm visually that the sample was homogenized adequately. The individual homogenized filets were combined and homogenized again to form a single batch (a “composite” sample) of pureed fish of one grade. Six 50 g samples of the homogenized fish of each grade were used for the method of standard addition experiment. The samples were placed in sealed mason jars allowing for head-space accumulation.

### Preparation of Bromophenol Blue (BPB) Strips

A solution was prepared by dissolving 1% bromophenol blue (BPB) (Acros Organics, Geel, Belgium) in 70% aqueous ethanol (Fischer Scientific, Pittsburgh, PA). One-inch squares of filter paper were soaked in this solution for one minute before being allowed to dry for an hour. These strips were used to measure the volatile amine content of the fish samples.

### Colorimetric Evaluation – BPB Strips

The sample exposed BPB indicator strips were measured using a colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan) on the  $L^*a^*b^*$  scale. Unexposed indicator strips were also measured using the colorimeter as the control to the exposed strips. The difference in  $b^*$  value between the unexposed (control) and exposed strips was calculated. Using the biogenic amine standard curve (Figure 1), the ppm of volatile biogenic amines in the samples were detected and calculated.

### Biogenic Amine Standard Curve – BPB Strips

A biogenic amine standard curve was created using the biogenic amine standard cocktail (histamine, cadaverine, tyramine, dimethylamine, trimethylamine) of four different concentrations (0, 10, 15, 20 and 50 ppm) in Milli-Q water (EMD Millipore Synergy Ultrapure Water Systems, Darmstadt, Germany). The BPB strips were exposed to the solutions for 60 minutes in a sealed jar. The  $b^*$  value difference between the unexposed BPB indicator strips and the exposed BPB strips was calculated to produce a standard curve to confirm that the response of the aqueous biogenic amine solutions increased with concentration (Figure 1).

### BPB Method of Standard Addition (MSA) Procedure

A biogenic amine standard cocktail was made with equal concentrations of histamine (Sigma-Aldrich, St Louis, MO), cadaverine (Fluka, Switzerland), tyramine (Acros Organics, Geel, Belgium), dimethylamine (Acros Organics, Geel, Belgium), and

trimethylamine (Acros Organics, Geel, Belgium). Three different concentrations of this cocktail were used: 10 ppm, 20 ppm, and 50 ppm. The cocktail was created and diluted with Milli-Q water. Duplicate samples were run for each concentration of standard cocktail. The spike cocktail was mixed with the sample fish in a 1:1 ratio (50 g fish to 50 mL spike solution). The unspiked sample (initial reading) was 50 g of homogenized fish diluted with 50 mL of Milli-Q water. Next, the BPB indicator strips were added to the jars. The samples were heated in  $45^{\circ}\text{C}$  water bath (Isotemp 220, Fischer Scientific, Pittsburgh, PA) for 60 minutes. The calculated  $b^*$  value was graphed against the ppm of standard cocktail spike for each grade. A standard addition plot was used to determine the concentration of biogenic amines in each unspiked sample by extrapolation of the equation of the line to the x-axis.

### Histamine ELISA Method of Standard Addition (MSA) Procedure

The AOAC (No. 070703) validated Neogen Veratox<sup>®</sup> test kit (Neogen Corp, Lansing, MI) is a histamine-specific ELISA test that was utilized in this study. In accordance with the kit instructions, 10 g samples of tuna or mahi-mahi were diluted with a 10 mL spike of each standard cocktail concentration and 80 mL of Milli-Q water. The procedure was performed in duplicate for each spike

concentration. The standard solutions provided with the Veratox ® kit and standard biogenic amines were used to construct separate standard curves. The concentration of histamine was calculated both by using the software provided by the manufacturer (Standard ELISA), and graphically using the equation of the line. The ELISA-MSA procedure using the equation of the line established the point where the line intersected the x-axis, thus giving the concentration of histamine in the unspiked sample. Unspiked samples (5 replications per grade) were also analyzed using the ELISA assay. The results of the ELISA assay given by the manufacturer software are reported to the third decimal place. Therefore, calculated histamine concentrations have four significant figures (Ebbing and Gammon, 1998).

## Results and Discussion

Two standard curves were created for the biogenic amine cocktail. Figure 1 shows the standard curve for the cocktail using the BPB strip method. The  $b^*$  score is the difference between the  $b^*$  values of the unexposed and exposed strips. The  $b^*$  value of the colorimeter represents a scale from yellow to blue; the more yellow hue present, the more positive the  $b^*$  value, alternatively the more blue hue present, the more negative the  $b^*$  value. With higher amine concentration, the BPB strips become more blue and as a result the  $b^*$  difference score (difference between the unexposed and exposed strips) increases as the concentration of volatile biogenic amines increases. Figure 2 shows the standard curve for the cocktail using the ELISA kit. Table 1 shows a comparison between the actual histamine concentration as part of the biogenic amine cocktail and the experimentally

determined histamine values by the ELISA kit assay. This was done to observe if there was any cross-reactivity with the other biogenic amines present in the cocktail. It should be noted that the actual histamine ppm represents the one-fifth portion of the overall cocktail concentration that is histamine. Multiple studies have shown that there are no significant cross-reactivity issues with the Neogen kit (Lupo and Mozola, 2011; Hungerford and Hu, 2012). The results of this study are consistent with these previous conclusions.

When comparing Figures 1 and 2, the shapes of the two curves differ. Figure 2 shows a direct linear relationship between concentration of the cocktail and concentration of detected histamine. The graph in Figure 1 lacks this distinctive shape; and the response is possibly an exponential relationship. This could be due to a number of reasons. First is the quantitative limit for the colorimeter and the strips themselves (the ability to differentiate between two sets of values). As the concentration of the cocktail approaches 50 ppm, one of two situations is likely occurring: the strips have reacted completely and can no longer change to a more intense blue color or the colorimeter has reached its detector saturation limit for the  $b^*$  value (the ability to measure a very negative  $b^*$  value). Therefore, it may have been possible the colorimeter could not accurately measure the pronounced blue color produced by a higher volatile amine concentration. Dye impregnated membranes have previously been used to indicate freshness in other products (Kuswandi, 2013). This type of technology is established, but quantitation issues many have limited its application in this particular situation.

**Table 1.** Expected vs. Actual Results of ELISA Cocktail Standard Curve.

Cocktail Concentration (ppm)	Actual Histamine Concentration (ppm)	Experimentally Determined Histamine – Standard ELISA (ppm)
10	2	3.894
15	3	5.496
20	4	7.358
50	10	15.91

**Table 2.** Mahi-Mahi Volatile Biogenic Amine and Histamine Results.

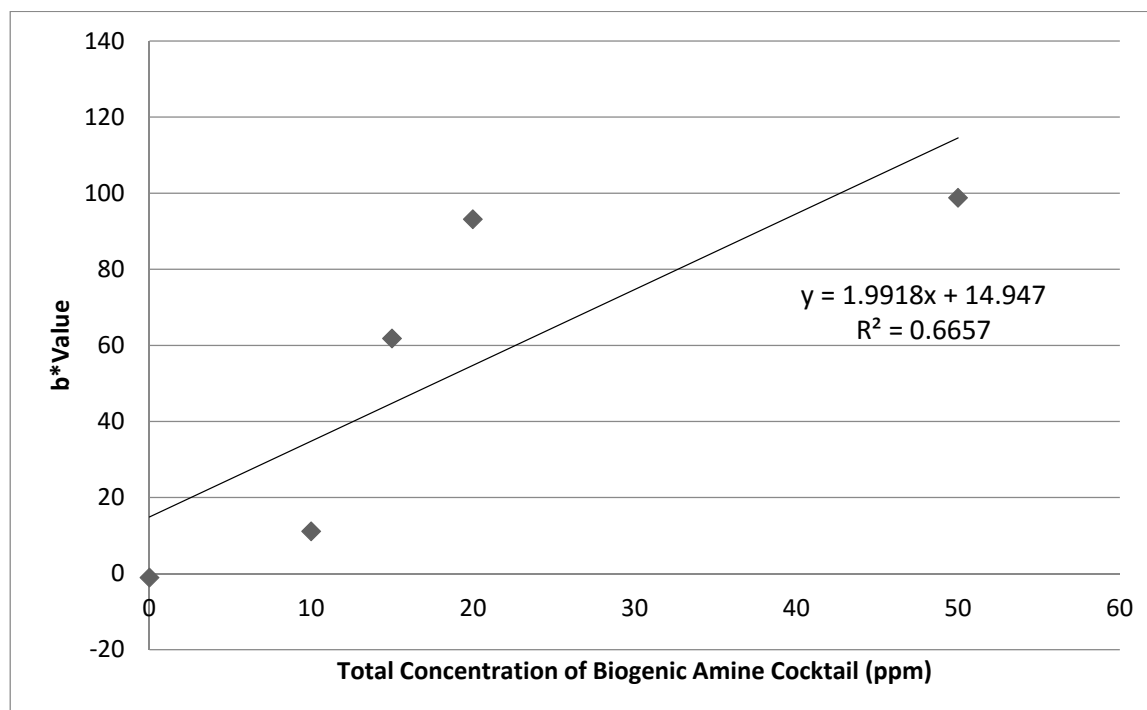
Grade	Volatile Biogenic Amines (ppm) – BPB-MSA	Histamine (ppm) – ELISA-MSA	Histamine (ppm) – Standard ELISA
M-1	35.17	3.389	8.399 ± 2.9
M-2	34.67	0.4086	0.65 ± 0.20
M-3	81.49	0.7989	3.690 ± 1.5
M-4	41.83	0.3830	1.261 ± 0.23
M-5	33.53	0.9583	1.438 ± 0.32
M-7	99.05	24.21	155.3 ± 20

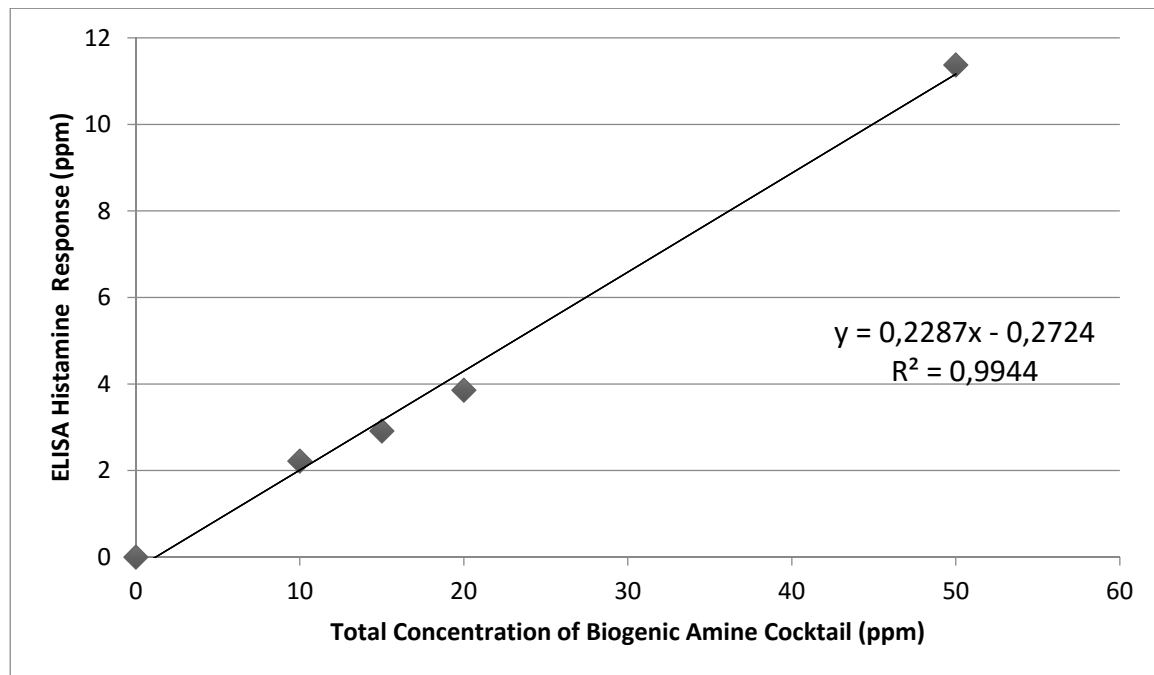
\* ± Standard error (SE) is reported only for the Standard ELISA procedure since an MSA procedure does not permit the computation of SE.

**Table 3.** Tuna Volatile Biogenic Amine and Histamine Results.

Grade	Volatile Biogenic Amines (ppm) – BPB-MSA	Histamine (ppm) – ELISA-MSA	Histamine (ppm) – Standard ELISA
T-1	25.37	0.0867	0.2362 ± 0.072
T-2	256.0	0.0130	0.1329 ± 0.13
T-3	58.60	0.0663	0.789 ± 0.27
T-4	111.0	0.0753	0 ± 0.0
T-5	194.6	5.239	81.99 ± 28
T-6	44.50	5.090	197.7 ± 71
T-7	27.10	0.5320	36.09 ± 6.8

\* ± Standard error (SE) is reported only for the Standard ELISA procedure since an MSA procedure does not permit the computation of SE.

**Figure 1.** Biogenic Amine Cocktail Standard Curve – BPB Strips.



**Figure 2.** Biogenic Amine Cocktail Standard Curve – ELISA Assay.

The class and phase of analyte being measured can account for these differences. The BPB strips measure gas phase biogenic amines while the ELISA kit measures liquid phase histamine. Ashie (1996) showed that a number of compounds, including trimethylamine, make up the volatile aroma mixture given off by fish. This is part of the reason why the standard cocktail used in this study contains more than just histamine and why the strips can be considered a non-specific assay. Because the grading of the fish is performed via olfactory detection, multiple volatile compounds were needed to best formulate a legitimate spiking solution. The ELISA assay, however, measures a specific compound, rather than a class of compounds. The phase of detection of this assay also could impact its effectiveness in this situation. Rather than the analyte being driven off by heat, as is the case with the BPB strip method, the histamine is directly extracted in the liquid phase from the fish, yielding a more direct means of detection.

The differences in the target analyte and detection method are likely responsible for the difference in the two standard curves when comparing the two assays. Given the nature of the MSA methodology, linearity of the graph is important for providing an accurate calculation of given analyte. When comparing the two assays, BPB strips and ELISA, the ELISA assay produced a curve with better linearity and better curve fit (Figures 1 & 2). This is

likely due to the nature of the assay as discussed above and how it more accurately measures a specific compound (histamine) versus a class of compounds.

For the majority of the ELISA-MSA graphs used to calculate the histamine content in the unspiked samples, the linearity was ideal. Any poor linearity for the ELISA graphs may be due to the limit of quantitation with the Neogen assay. As the grades of samples approached the “fail” range, extra dilution steps were necessary to bring the sample back within the ideal detection range for histamine (2-40 ppm). The kit is more reliable within the manufacturer specified detection range; any samples analyzed that exceed this range (despite further dilution) are more likely to be inaccurately measured.

Graph linearity and the limit of quantitation of the assays need to be taken into account when analyzing the calculated ELISA-MSA values in the right hand columns of Tables 2 and 3. The more accurately calculated concentrations are for Grades 1-5 in Table 2 given the linearity of the graphs that were produced. Similarly, the calculated values in Table 3 are also likely more accurate for the “pass” grades. This trend for both mahi-mahi and tuna samples demonstrates the limit for quantitation for the ELISA assay.

In Table 3, we see an anomaly with the Grade 7 tuna: it has a much lower calculated histamine

concentration than the other two “fail” grades (Grades 5 and 6). This could be caused by an initial concentration that exceeded the detection range of the assay. The Grade 7 tuna samples underwent 3 extra dilutions to put it in the range of quantitation, more than any other sample grade. The limit of quantitation may explain the trend shown in Table 3. As for Table 2, Grade 1 of the mahi-mahi has higher histamine concentration than any other grades excluding Grade 7.

Both Tables 2 and 3 show the results of the ELISA-MSA and standard ELISA procedures. When comparing the standard ELISA values to the ELISA-MSA values, most of the standard ELISA values are higher than those found in the ELISA-MSA procedure. This may be attributed to the methodology of how the ELISA-MSA (spiked) concentrations were calculated and demonstrates why linearity is a major factor in producing accurate results with the MSA assay.

Overall, with both ELISA assays, greater histamine concentrations are seen in lower quality samples (e.g. Grades 5, 6 and 7) as expected. In comparison, there is no true trend with the BPB-MSA data. Lack of a linear increase of analyte as spoilage increases may be attributed to the poor linearity of the graphs used to calculate the volatile biogenic amine contents, which relates back to the overall matrix effect. Others have reported the less linear the MSA graph is, the stronger the effect of the matrix or other interfering compounds that inhibit the selectivity of the matrix (Saxberg, 1979). When comparing the different natures of the two assays and the results derived from them, it can be deduced that the matrix effect is much more prominent for gaseous phase (volatile) compounds than liquid phase compounds.

The BPB-MSA results may be explained due to the nature of volatile compounds: volatile compounds tend to be lipophilic (lack hydrophobicity) and therefore more likely to be bound in the flesh than present in the gas phase (Druaux and Voilley, 1997). Comparing the MSA and ELISA methodologies demonstrates this principle. The data for the ELISA (liquid-phase) assay followed more of an expected pattern than for the BPB strips (gaseous-phase assay) likely due to the fact that there was more of the histamine present in the flesh (histamine is non-volatile), not bound by protein (histamine is formed from unbound histidine) and present mostly in the lipid portion of the fish. The results reported by Lerke (1978) demonstrated that histamine is found in highest concentrations in the

lipid portion near the gut cavity. Both free histidine and histamine is found in greater concentration in the fattier red muscle tissue (Hultin, 1984; Lukton and Olcott, 1958; Takagi et al., 1969). A major concern as fish spoil is protease breakdown, which leads to muscle softening (Hultin, 1984). Bacterial spoilage both directly causes formation of histamine (Yamanaka, 1982) while also indirectly breaking down protein through the formation of trimethylamine oxide (Hultin, 1984). The bacterial breakdown of the protein portion of the fish by the protein creates a positive feedback loop as it provides an ideal environment for further microbiological growth (Lerke, 1967). The breakdown of the flesh through spoilage, and release of biogenic amines that results, likely overwhelms the BPB assay and leads to poor results for lower quality (higher grade) samples.

## Conclusion

The ELISA-MSA results demonstrated an increase in histamine content as the quality of either tuna or mahi-mahi decreased. A comparison of the two assays (BPB strips and ELISA) showed that the matrix effect was much stronger for volatile compounds than aqueous compounds. The lipophilic nature of aromatic compounds helps partially account for the discrepancy between the results of the two MSA assays. The ELISA assay seemed to be better adept at extracting and detecting the desired compounds, it provided a more accurate representation of the trend in the fish samples; that is, histamine increases as quality decreases. Given these conclusions, the sensory grading system does represent what it intends to: a higher quality fish being safer to consume. However, the fact that these grades are based upon an orthonasal panel draws into question how accurate orthonasal measurement is for fish when there seems to be such a strong matrix effect with the volatile compounds. Further research should determine what proportion of volatile spoilage indicators (biogenic amines in this case) are present in the liquid and gaseous phase at each grade to assure that grading accurately reflects the safety of the product.

## References

- Ahmed, F.E. (1991). Naturally occurring fish and shellfish poisons. *National Academy Press*, 87,110.
- Altaki, M.S., Santos, FJ. & Galceran, MT. (2007). Analysis of furan in foods by headspace

Journal abbreviation: **J Food Health Sci**

- solid-phase microextraction-gas chromatography-ion trap mass spectrometry. *Journal of Chromatography*, 1146(1), 103-109.
- AOAC-RI No. 070703. (2005). Retrieved from <http://www.neogen.com/Food-Safety/pdf/ProdInfo/V-Hist.pdf> (accessed 12.28.2015)
- Ashie, I.N.A., Smith, J.P., Simpson, B.K. & Haard, N.F. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition*, 36(1-2), 87-121.
- [CDC] Centers for Disease Control and Prevention. (2013). Food poisoning from marine toxins. Retrieved from [www.cdc.gov](http://www.cdc.gov) (accessed 01.20.2015)
- Druaux, C. & Voilley, A. (1997). Effect of food composition and microstructure on volatile flavor release. *Trends in Food Science & Technology*, 8(11), 364-368.
- Ebbing, DD. & Gammon, SD. (1998). *General chemistry 6<sup>th</sup> ed.* New York: Houghton Mifflin Company.
- [FDA] Food and Drug Administration. (2013). ORA laboratory manual – volume IV – section 8 – sensory analysis. Document no. IV-08, version 1.4, 1(15). Retrieved from <http://www.fda.gov/ScienceResearch/Field-Science/LaboratoryManual/ucml72190.htm> (accessed 02.10.2016)
- Halász, A., Baráth, A., Simon-Sarkadi, L. & Holzapfel W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology*, 5, 42-49.
- Hidalgo, F.J., Navarro, J.L., Delgado, R.M. & Zamora, R. (2013). Histamine formation by lipid oxidation products. *Food Research International*, 52, 206-213.
- Hultin, H.O. (1984). Postmortem biochemistry of meat and fish. *Journal of Chemical Education*, 61(4), 289-298.
- Hungerford, J. & Wu, W.H. (2012). Comparison study of three rapid test kits for histamine in fish: bioscientific maxsignal enzymatic assay, neogen veratox ELISA, and the neogen reveal histamine screening test. *Food Control*, 25, 448-457.
- Kuswandi, B., Maryska, C., Jayus, Abdullah, A. & Heng, L.Y. (2013). Real time on-package freshness indicator for guavas packaging. *Journal of Food Measurement and Characterization*, 7(1), 29-39.
- Lerke, P., Farber, L. & Adams, R. (1967). Bacteriology of spoilage of fish muscle IV role of proteins. *Applied Microbiology*, 15(4), 770-776.
- Lerke, P.A., Werner, B., Taylor, S.L. & Guthertz LS. (1978). Scombroid poisoning – report of an outbreak. *Western Journal of Medicine*, 129(5), 381-386.
- Lukton, A. & Olcott, H.S. (1958). Content of free imidazole compounds in the muscle tissue of aquatic animals. *Food Research*, 23, 611-618.
- Lupo, A. & Mozola, M. (2011). Validation study of a rapid ELISA for detection of histamine in tuna. *Journal of AOAC International*, 94(3), 886-899.
- Pinho, O., Ferreira, I.M.P.L.V.O. & Merreira, M.A. (2002). Solid-phase microextraction in combination with GC/MS for quantification of the major volatile free fatty acids in ewe cheese. *Analytical Chemistry*, 74, 5199-5204.
- Pivarnik, L., Ellis, P., Wang, X. & Reilly, T. (2001). Standardization of the ammonia electrode method for evaluating seafood quality by correlation to sensory analysis. *Journal of Food Science*, 66(7), 945-952.
- Prester, L. (2011). Biogenic amines in fish, fish products, and shellfish: a review. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 28(11), 1547-1560.
- Saxberg, B.E.H. & Kowalski, B.R. (1979). Generalized standard addition method. *Analytical Chemistry*, 51(7), 1031-1038.
- Takagi, M., Iida, A., Murayama, H. & Soma, S. (1969). On the formation of histamine during loss of freshness and putrefaction of various marine products. *Hokkaido Daigaku Suisan Gakubu Kenkyu Iho*, 20, 227-234.
- Yamanaka, H., Shiomi, K., Kikuchi, T. & Okuzumi, M. (1982). A pungent compound produced in the meat of frozen yellowfin tuna and marlin. *Bulletin of the Japanese Society of Scientific Fisheries*, 48(5), 685-689.